Testing the homocysteine hypothesis in end-stage renal disease: Problems and a possible solution

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The homocysteine hypothesis states that circulating homocysteine is a vascular toxin in concentrations that occur in the general population and in renal failure. This hypothesis is currently being tested in the Kidney and End State Renal Disease Study (HOST), but data have emerged since the HOST began that suggest its results will be inconclusive. The crucial treatment component in the HOST is folic acid, but its effect is likely to be lost because the American food supply is now fortified with folic acid. A second concern is that the very high doses of folic acid and pyridoxine being used in the HOST may confound the results. Finally, confounding due to 'reverse epidemiology' was not considered when the HOST was designed. Parenteral vitamin B_{12} is a highly promising therapy for homocysteine reduction in end-stage renal disease that merits careful investigation. Clinical trials using it to test the homocysteine hypothesis will avoid the problems inherent in the HOST.

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There is little doubt that in sufficiently high concentrations homocysteine is a vascular toxin. In untreated homocystinuria, plasma total homocysteine (tHcy) concentrations range between 100 and 400 μmol/l; unless these are lowered by vitamin therapy, patients die at a young age from venous thromboembolism and malignant arterial disease.[1–4] Hyperhomocysteinemia causes arterial disease in experimental animals [5–8] by a variety of plausible mechanisms.[3,9–12] The important question – the 'homocysteine hypothesis' – is whether homocysteine is toxic in concentrations that occur in the general population (5–15 μmol/l) or in advanced and end-stage renal disease (ESRD) (15–50 μmol/l), and whether lowering these concentrations will slow the progression of cardiovascular disease.[13]

Most epidemiologic studies have shown a strong and graded association between the risk of cardiovascular events and plasma tHcy concentrations as low as 10 μmol/l.[1,14–16] Some inconsistencies that have been reported in this relationship may be reconciled if other vascular risk factors are required to be present for mild hyperhomocysteinemia to be toxic.[17–19] This interaction would also account for the effectiveness of vitamin therapy in preventing vascular complications in vitamin-responsive homocystinuria despite residual mild hyperhomocysteinemia.[4]

Ultimately, proof of the homocysteine hypothesis will require convincing results from hard end point randomized clinical trials.[20] In a prospective randomized clinical trial in Switzerland, 6 months of daily therapy with 1 mg folic acid, 0.4 mg vitamin B_{12}, and 10 mg pyridoxine lowered the average plasma tHcy concentration from 11.4 to 7.5 μmol/l and substantially reduced the need for revascularization in coronary angioplasty patients, especially those treated by balloon angioplasty.[21–23] In a different study from the Netherlands, daily therapy with 1.2 mg folic acid, 60 μg vitamin B_{12}, and 48 mg pyridoxine paradoxically increased the incidence of coronary restenosis in patients treated by intracoronary stenting, despite lowering the average plasma tHcy concentration from 12.2 to 9.0 μmol/l.[24]

In the Vitamin Intervention for Stroke Prevention (VISP) study, 3680 Americans and Canadians with a recent stroke were randomized to a daily regimen of 2.5 mg folic acid, 0.4 mg vitamin B_{12}, and 25 mg pyridoxine or control tablets.[25] This treatment lowered the average plasma tHcy concentration only slightly from 13.4 to 11 μmol/l, and had

no detectable effect on vascular outcomes during the 2 year follow-up period. The VISP study began just as grain products in the American and Canadian diet were fortified with synthetic folic acid. This intervention lowered plasma tHcy concentrations in the general population,[26] diminishing the effect of folic acid in the therapeutic arm of the VISP and other studies.[27,28] The diet is not fortified with folic acid in Europe, and in a clinical trial recently completed there 50 patients with increased carotid intima-media thickness were randomized to the same vitamin regimen as in the VISP study.[29] Vitamin therapy lowered the average plasma tHcy concentration from 10.5 to 6.6 μmol/l, more than twice as much as in the VISP study, and was associated with a significantly lower carotid intima-media thickness.[29]

Chronic renal failure and ESRD are important conditions in which to test the homocysteine hypothesis.[30] If the homocysteine hypothesis were to be confirmed in this population, it would increase the likelihood that homocysteine lowering at an early stage of renal disease could slow its progression.[31] The Homocysteinemia in Kidney and End State Renal Disease Study (HOST) is a US Veterans Administration–industry funded clinical trial initiated in 2001 to determine the effect of 4 years of a daily regimen of 40 mg folic acid, 100 mg pyridoxine, and 2 mg of vitamin B_{12} on vascular outcomes in persons with advanced chronic renal failure and ESRD (http://clinicaltrials.gov/ct/show/NCT00032435). Since 2001 data have emerged that strongly suggest the HOST will be inconclusive.

Folic acid is the key therapeutic component in the HOST, but, as demonstrated by the VISP study, the homocysteine-lowering effect of folic acid is likely to be lost because the American food supply is now fortified. The 40 mg dose of folic acid being used in HOST is no more effective than 1 mg/day at reducing plasma tHcy concentrations[32–35] and may create confusion, since high-dose folic acid could have a therapeutic benefit independent of plasma homocysteine lowering.[36] Thus, even a positive outcome of the HOST would leave the homocysteine hypothesis untested.

A second problem is the use of pyridoxine in dose more than 60 times the recommended intake of 1.5 mg/day. The only noteworthy design difference between the Swiss and Netherlands angioplasty trials is the extremely high pyridoxine dose used in the Netherlands study, in which active treatment led to an adverse outcome.[24] This result raises the disturbing possibility that very-high dose pyridoxine could be toxic in some situations. Pyridoxine is neurotoxic in very high doses.[3] The active form of the vitamin, pyridoxal 5′-phosphate, has a variety of biochemical actions, some of which are beneficial in short-term clinical studies;[37,38] but any biologically active molecule could have potentially harmful long-term effects in very high doses, particularly in the absence of a mitigating benefit such as homocysteine lowering. The extremely high folic acid and pyridoxine doses being used in the HOST are biochemically unjustified. Both vitamins reduce plasma tHcy concentrations by eliminating a deficiency state, and there is no good evidence that doses higher than necessary to eliminate deficiency cause additional plasma tHcy lowering, except in homocystinuria. High-flux dialyzers remove pyridoxal-5′-phosphate efficiently, but daily pyridoxine doses of 10, or at most 20 mg/day appear to compensate adequately for it.[39,40]

Third, any test of the homocysteine hypothesis must take into account the problem of 'reverse epidemiology.' Some ESRD patients have low plasma tHcy concentrations that are related to inflammatory states, malnutrition, and hypoalbuminemia, all of which are associated with a poor clinical outcome.[41–43] Approximately 70% of plasma tHcy circulates bound to albumin, so hypoalbuminemia lowers plasma tHcy concentrations without necessarily lowering concentrations of the metabolically active species, reduced homocysteine.[43] Patients with plasma tHcy concentration as low as 15 μmol/l are enrolled in the HOST. Reverse epidemiology could well apply to these patients. Another concern with enrolling such patients is that their plasma tHcy concentrations are most unlikely to decrease with treatment; their inclusion will merely dilute the results.[44]

An alternative and highly potent treatment is now available to reduce plasma tHcy in ESRD, namely, parenteral vitamin B_{12}. Observational data[45,46] motivated us to carry out a randomized clinical trial comparing the effect of standard high-dose oral folic acid, pyridoxine, and vitamin B_{12} with the same regimen to which 1 mg hydroxocobalamin was administered parenterally after dialysis once per week. This addition lowered plasma tHcy concentrations by an average of 32%.[47] Almost simultaneously, a controlled trial by Koyama *et al.*[48] demonstrated an even greater, 50% reduction in plasma tHcy by the addition of 0.5 mg intravenous methylcobalamin after every dialysis to a high-dose folic acid-pyridoxine regimen. We have now published two additional confirmatory controlled trials in different hemodialysis patient populations,[49,50] the most recent of which showed that 1 mg/day folic acid is sufficient for folate repletion, and that intravenous cyanocobalamin and hydroxocobalamin are equipotent even though they increase serum cobalamin levels differently.[50] Three other centers have now reported that a vitamin combination which includes parenteral vitamin B_{12} lowers plasma tHcy concentrations of hemodialysis patients to normal or nearly normal.[51–53] Since massive daily oral vitamin therapy (including oral vitamin B_{12}) lowers ESRD plasma tHcy by no more than one-third,[54–58] these reports represent further confirmation that parenteral vitamin B_{12} is the critical component responsible for normalizing plasma tHcy concentrations in folate- and vitamin B_{12}-replete ESRD patients.

The cause of renal failure-associated hyperhomocysteinemia continues to be debated.[59,60] Reduced renal mass likely plays a role since the kidney contains enzymes for metabolizing homocysteine and cysteine, and the concentrations of both amino acids are increased in renal failure.[45,61] The clinical reports now available from several centers provide strong inferential evidence that uremia induces hyperhomocysteinemia by inhibiting *methionine synthase*,

the cobalamin-dependent enzyme that catalyzes homocysteine remethylation to methionine. Pharmacologic concentrations of cobalamin are known to increase *methionine synthase* activity many-fold via a post-translational mechanism.[62] Cobalamin must be administered parenterally because, unlike folic acid or pyridoxine, its oral bioavailability is too limited to raise tissue concentrations to the required pharmacologic level.[46] This novel understanding of cobalamin metabolism in uremia is in line with an earlier suggestion by van Guldener *et al.*,[63] who concluded from a clinical tracer study that homocysteine remethylation is impaired in hemodialysis patients.

It is appropriate to confirm the homocysteine-lowering effect of parenteral cobalamin in ESRD in additional centers (preferably the potent regimen of 0.5 mg intravenously after every dialysis used by Koyama *et al.*[48]) without waiting for the results of the HOST. Cyanocobalamin, an inexpensive and widely available form of the vitamin used in North America, is probably equipotent to the methylcobalamin used by Koyama *et al.*[48] There are major advantages to testing a regimen such as 1 mg oral folic acid, 10 or 20 mg oral pyridoxine, and 0.5 mg intravenous cobalamin after every dialysis. This regimen will avoid potentially serious confounding due to high-dose folic acid and pyridoxine as used in the HOST. The lowering of plasma tHcy can be anticipated to be from approximately 25 to 12 μmol/l, which is sufficiently great to permit a study that enrolls many fewer patients than in the HOST. To avoid confounding due to reverse epidemiology, patients with hypoalbuminemia, malnutrition, or a plasma tHcy less than $\sim$20 μmol/l should be excluded from studies of the homocysteine hypothesis in ESRD.

REFERENCES

1. Nygard O, Vollset SE, Refsum H *et al.* Total homocysteine and cardiovascular disease. *J Intern Med* 1999; **246**: 425–454.
2. Bostom AG, Selhub J. Homocysteine and arteriosclerosis: subclinical and clinical disease associations. *Circulation* 1999; **99**: 2361–2363.
3. Mudd SH, Levy HL, Kraus JP. Disorders of transsulfuration. In: Scriver CR, Beaudet A, Sly WS, Valle D (eds). *The Metabolic & Molecular Bases of Inherited Disease*, 8 edn., McGraw-Hill: New York, 2001, pp 2007–2056.
4. Yap S. Classical homocystinuria: vascular risk and its prevention. *J Inher Metab Dis* 2003; **26**: 259–265.
5. McCully KS, Wilson RB. Homocysteine theory of arteriosclerosis. *Atherosclerosis* 1975; **22**: 215–227.
6. Mohammed R, Lamand M. Cardiovascular lesions in cobalt-vitamin B12 deficient sheep. *Ann Rech Vet* 1986; **17**: 447–450.
7. Hofmann MA, Lalla E, Lu Y *et al.* Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. *J Clin Invest* 2001; **107**: 675–683.
8. Symons JD, Mullick AE, Ensunsa JL *et al.* Hyperhomocysteinemia evoked by folate depletion: effects on coronary and carotid arterial function. *Arterioscler Thromb Vasc Biol* 2002; **22**: 772–780.
9. Durand P, Lussier-Cacan S, Blache D. Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats. *FASEB J* 1997; **11**: 1157–1168.
10. Zhang X, Li H, Jin H *et al.* Effects of homocysteine on endothelial nitric oxide production. *Am J Physiol* 2000; **279**: F671–F678.
11. Perna AF, Acanfora F, Satta E *et al.* Hyperhomocysteinemia and cardiovascular disease in uremia: the newest evidence in epidemiology and mechanisms of action. *Semin Nephrol* 2004; **24**: 426–430.
12. Wilson KM, Lentz SR. Mechanisms of the atherogenic effects of elevated homocysteine in experimental models. *Semin Vasc Med* 2005; **5**: 163–171.
13. McCully KS. Homocysteine and vascular disease. *Nat Med* 1996; **2**: 386–389.
14. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 1999; **99**: 178–182.
15. Eikelboom JW, Lonn E, Genest Jr J *et al.* Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* 1999; **131**: 363–375.
16. Goldstein LB, Adams R, Becker K *et al.* Primary prevention of ischemic stroke: a statement for healthcare professionals from the Stroke Council of the American Heart Association. *Circulation* 2001; **103**: 163–182.
17. Refsum H, Ueland PM. Recent data are not in conflict with homocysteine as a cardiovascular risk factor. *Curr Opin Lipidol* 1998; **9**: 533–539.
18. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000; **72**: 324–332.
19. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. *Thromb Haemostasis* 1999; **81**: 165–176.
20. Splaver A, Lamas GA, Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. *Am Heart J* 2004; **148**: 34–40.
21. Schnyder G, Roffi M, Pin R *et al.* Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med* 2001; **345**: 1593–1600.
22. Schnyder G, Roffi M, Flammer Y *et al.* Effect of homocysteine-lowering therapy with folic acid, vitamin B12, and vitamin B6 on clinical outcome after percutaneous coronary intervention. *JAMA* 2002; **288**: 973–979.
23. Schnyder G, Roffi M, Flammer Y *et al.* Effect of homocysteine-lowering therapy on restenosis after percutaneous coronary intervention for narrowings in small coronary arteries. *Am J Cardiol* 2003; **91**: 1265–1269.
24. Lange H, Suryapranata H, De L *et al.* Folate therapy and in-stent restenosis after coronary stenting. *N Engl J Med* 2004; **350**: 2673–2681.
25. Toole JF, Malinow MR, Chambless LE *et al.* Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004; **291**: 565–575.
26. Pfeiffer CM, Caudill SP, Gunter EW *et al.* Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. *Am J Clin Nutr* 2005; **82**: 442–450.
27. Bostom AG, Selhub J, Jacques PF, Rosenberg IH. Power shortage: clinical trials testing the 'homocysteine hypothesis' against a background of folic acid-fortified cereal grain flour. *Ann Intern Med* 2001; **135**: 133–137.
28. Bostom AG, Jacques PF, Liaugaudas G *et al.* Total homocysteine lowering treatment among coronary artery disease patients in the era of folic acid-fortified cereal grain flour. *Arterioscler Thromb Vasc Biol* 2001; **22**: 488–491.
29. Till U, Rohl P, Jentsch A *et al.* Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, Vitamins B6 and B12. *Atherosclerosis* 2005; **181**: 131–135.
30. Austen SK, Coombes JS, Fassett RG. Homocysteine and cardiovascular disease in renal disease. *Nephrology* 2003; **8**: 285–295.
31. Hebert LA, Wilmer WA, Falkenhain ME *et al.* Renoprotection: one or many therapies? *Kidney Int* 2001; **59**: 1211–1226.
32. Sunder-Plassmann G, Fodinger M, Buchmayer H *et al.* Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna multicenter study. *J Am Soc Nephrol* 2000; **11**: 1106–1116.
33. Homocysteine Lowering TC. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr* 2005; **82**: 806–812.
34. Gonin JM, Nguyen H, Gonin R *et al.* Controlled trials of very high dose folic acid, vitamins B_{12} and B_6, intravenous folinic acid and serine for treatment of hyperhomocysteinemia in ESRD. *J Nephrol* 2003; **16**: 522–534.
35. Wrone EM, Hornberger JM, Zehnder JL *et al.* Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. *J Am Soc Nephrol* 2004; **15**: 420–426.
36. Doshi SN, Moat SJ, McDowell IF *et al.* Lowering plasma homocysteine with folic acid in cardiovascular disease: what will the trials tell us? *Atherosclerosis* 2002; **165**: 1–3.
37. Wang X, Dakshinamurti K, Musat S, Dhalla NS. Pyridoxal 5′-phosphate is an ATP-receptor antagonist in freshly isolated rat cardiomyocytes. *J Mol Cell Cardiol* 1999; **31**: 1063–1072.
38. Kandzari DE, Dery JP, Armstrong PW *et al.* MC-1 (pyridoxal 5′-phosphate): novel therapeutic applications to reduce ischaemic injury. *Expert Opin Investig Drugs* 2005; **14**: 1435–1442.

39. Mydlik M, Derzsiova K, Zemberova E. Metabolism of vitamin B_6 and its requirement in chronic renal failure. *Kidney Int* 1997; **62**: S56–S59.
40. Kasama R, Koch T, Canals-Navas C, Pitone JM. Vitamin B6 and hemodialysis: the impact of high-flux/high-efficiency dialysis and review of the literature. *Am J Kidney Dis* 1996; **27**: 680–686.
41. Kalantar-Zadeh K, Block G, Humphreys MH, Kopple JD. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int* 2003; **63**: 793–808.
42. Suliman ME, Stenvinkel P, Qureshi AR *et al.* Hyperhomocysteinemia in relation to plasma free amino acids, biomarkers of inflammation and mortality in patients with chronic kidney disease starting dialysis therapy. *Am J Kidney Dis* 2004; **44**: 455–465.
43. Suliman ME, Barany P, Kalantar-Zadeh K *et al.* Homocysteine in uraemia – a puzzling and conflicting story. *Nephrol Dial Transplant* 2005; **20**: 16–21.
44. Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin intervention for stroke prevention trial: an efficacy analysis. *Stroke* 2005; **36**: 2404–2409.
45. Hoffer LJ, Bank I, Hongsprabhas P *et al.* A tale of two homocysteines – and two hemodialysis units. *Metabolism* 2000; **49**: 215–219.
46. Hoffer LJ. Renal failure, homocysteine, and the pharmacology of vitamin B_{12}. *Metabolism* 2002; **51**: 399–402.
47. Elian KM, Hoffer LJ. Hydroxocobalamin reduces hyperhomocysteinemia in end-stage renal disease. *Metabolism* 2002; **51**: 881–886.
48. Koyama K, Usami T, Takeuchi O *et al.* Efficacy of methylcobalamin on lowering total homocysteine plasma concentrations in haemodialysis patients receiving high-dose folic acid supplementation. *Nephrol Dial Transplant* 2002; **17**: 916–922.
49. Hoffer LJ, Saboohi F, Golden M, Barre PE. Cobalamin dose regimen for maximum homocysteine reduction in end-stage renal disease. *Metabolism* 2005; **54**: 835–840.
50. Hoffer LJ, Djahangirian O, Bourgouin PE *et al.* Comparative effects of hydroxocobalamin and cyanocobalamin on plasma homocysteine concentrations in end-stage renal disease. *Metabolism* 2005; **54**: 1362–1367.
51. Sombolos K, Fragia T, Natse T *et al.* The effect of long-term intravenous high dose B-complex vitamins with or without folic acid on homocysteine in hemodialysis patients. *J Nephrol* 2002; **15**: 671–675.
52. Baragetti I, Furiani S, Dorighet V *et al.* Effect of vitamin B_{12} on homocysteine plasma concentration in hemodialysis patients. *Clin Nephrol* 2004; **61**: 161–162.
53. Obeid R, Kuhlmann MK, Kohler H, Herrmann W. Response of homocysteine, cystathionine, and methylmalonic acid to vitamin treatment in dialysis patients. *Clin Chem* 2005; **51**: 196–201.
54. Bostom AG, Shemin D, Lapane KL *et al.* High dose-B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 1996; **49**: 147–152.
55. Moustapha A, Gupta A, Robinson K *et al.* Prevalence and determinants of hyperhomocysteinemia in hemodialysis and peritoneal dialysis. *Kidney Int* 1999; **55**: 1470–1475.
56. Bostom AG, Shemin D, Bagley P *et al.* Controlled comparison of L-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Circulation* 2000; **101**: 2829–2832.
57. Manns B, Hyndman E, Burgess E *et al.* Oral vitamin B_{12} and high-dose folic acid in hemodialysis patients with hyper-homocyst(e)inemia. *Kidney Int* 2001; **59**: 1103–1109.
58. van Tellingen A, Grooteman MC, Bartels PM *et al.* Long-term reduction of plasma homocysteine levels by super-flux dialyzers in hemodialysis patients. *Kidney Int* 2001; **59**: 342–347.
59. Friedman AN, Bostom AG, Selhub J *et al.* The kidney and homocysteine metabolism. *J Am Soc Nephrol* 2001; **12**: 2181–2189.
60. van Guldener C, Stehouwer CD. Homocysteine and methionine metabolism in renal failure. *Semin Vasc Med* 2005; **5**: 201–208.
61. Suliman ME, Anderstam B, Lindholm B, Bergstrom J. Total, free, and protein-bound sulphur amino acids in uraemic patients. *Nephrol Dial Transplant* 1997; **12**: 2332–2338.
62. Oltean S, Banerjee R. Nutritional modulation of gene expression and homocysteine utilization by vitamin B_{12}. *J Biol Chem* 2003; **278**: 20778–20784.
63. van Guldener C, Kulik W, Berger R *et al.* Homocysteine and methionine metabolism in ESRD: a stable isotope study. *Kidney Int* 1999; **56**: 1064–1071.